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Uniform Program Standards for the Voluntary Bovine Johne's Disease Control Program

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Introduction

This document describes the cooperative State–Federal–Industry Voluntary Bovine Johne’s Disease Control Program to be administered by the State and supported by industry and the Federal Government. This publication is intended as a working document that will change as the program develops.

The objective of this program is to provide minimum national standards for the control of Johne’s disease. The program consists of three basic elements: (1) Education, to inform producers about the cost of Johne’s disease and to provide information about management strategies to prevent, control, and eliminate it; (2) Management, to work with producers to establish good management strategies on their farms; and (3) Herd Testing and Classification, to help separate test-positive herds from test-negative herds.

The program has been developed in cooperation with the National Johne’s Working Group and the Johne’s committee of the United States Animal Health Association, State Veterinarians, and industry representatives. The program has been approved by the U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Veterinary Services (VS).

The minimum national standards described in this document do not preclude the adoption of more stringent methods and rules by any geographical or political subdivision of the United States with regard to activities within their boundaries. However, regulations dealing with interstate movement must still conform to Federal regulations.

Part I Definitions and Abbreviations

Accredited veterinarian	A veterinarian approved by the APHIS Administrator in accordance with the provisions of Title 9 Code of Federal Regulations (CFR) Part 161 to perform functions required by State–Federal–Industry cooperative programs.
Administrator	The Administrator of APHIS or any person authorized to act for the Administrator.
Animal health official	A full-time employee of the State animal health department or of APHIS who has authority from the State Veterinarian or the Area Veterinarian-in-Charge (AVIC) to carry out program activities.
Anniversary date	The date on which the designated Johne's disease coordinator (DJC) gave final approval for initial program participation for a herd.
APHIS	Animal and Plant Health Inspection Service, an agency of the U.S. Department of Agriculture.
Approved laboratory	A private, State, Federal, or university laboratory that has passed an annual check test for Johne's disease administered by the National Veterinary Services Laboratories (NVSL). All program testing must be done in a laboratory approved by NVSL for the specific test being used in a State's testing program.
AVIC	Area Veterinarian-in-Charge: The veterinary official of Veterinary Services (VS), APHIS, USDA, assigned by the Administrator to supervise and perform the official animal health work of APHIS in the State or States concerned.
CFR	Code of Federal Regulations.
Commingling	Physical contact with susceptible species. For example, all cattle in the same pen, corral, or vehicle or all cattle grazed together on the same area of a property or farm will be considered commingled.
DJC	Designated Johne's disease coordinator: A person who has demonstrated the knowledge and ability to perform the functions required under these program standards and who has been selected for this position by the State animal health official and the AVIC. The VS regional Johne's disease epidemiologist or Regional Director and the VS Johne's disease staff must concur in the selection and appointment of the DJC.
ELISA	Enzyme-linked immunosorbent assay.
Exposure	Contact with known infected animals, contact with the manure or raw milk of infected or exposed animals of susceptible species, or contact with infected herds via contaminated water or feed sources.
Herd	A group of animals that has been managed as a separate and discrete unit. This may include two or more geographically separated groups of animals under common ownership or supervision but which have an interchange or movement of animals without regard to health status. The DJC will make the final determination of the herd status of a group of animals.

Herd management plan	A written plan, produced by the Johne's certified veterinarian or animal health official in conjunction with the producer, that includes animal husbandry and hygiene practices specific to that herd and that is designed to limit opportunities for exposure to <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> .
Herd member	An animal of any susceptible species that is commingled with the herd.
Infected animal	An animal that has been confirmed by an official Johne's disease test to be infected with <i>Mycobacterium avium</i> subsp. <i>paratuberculosis</i> .
Johne's certified veterinarian	An accredited veterinarian who has completed training approved by the DJC for Johne's disease epidemiology and development of herd management plans.
Johne's disease (JD)	An intestinal bacterial disease caused by <i>M. avium</i> subsp. <i>paratuberculosis</i> . Clinical signs, which appear after a long incubation period, include longlasting or chronic diarrhea and weight loss despite a good appetite.
Level achievement year	The year a herd in the Herd Testing and Classification element of the program obtained its current classification.
Management herd	A herd that has completed a risk assessment and herd management plan that satisfies the requirements of the DJC but has not completed the requirements for the test-negative or test-positive levels.
NVSL	National Veterinary Services Laboratories, APHIS laboratories in Ames, IA, and Plum Island, NY.
Official eartag	An identification eartag approved by APHIS as being tamper resistant and providing unique identification for each animal. An official eartag may conform to the alphanumeric National Uniform Eartagging System, or it may bear the valid premises identification used in conjunction with the producer's livestock production numbering system to provide a unique identification number.
Official Johne's disease test	An organism-detection test approved by the Administrator and conducted in a laboratory approved by the Administrator. The Administrator approves laboratories to conduct an official Johne's disease test only after determining that the laboratory meets the check-test proficiency requirements prescribed by NVSL. Approval continues as long as such check-test proficiency requirements are met on an annual basis.
Premises identification number	A unique number assigned by the State animal health official to a livestock production unit that is, in the judgment of the State animal health official and AVIC, epidemiologically distinct from other livestock production units. The premises' identification number consists of the State's two-letter postal abbreviation followed by the premises' assigned number or code.
Program	Voluntary Bovine Johne's Disease Control Program.

Screening test	A Johne's disease test approved by the Administrator for use in the Voluntary Bovine Johne's Disease Control Program and conducted in a laboratory validated through an approval process by NVSL. Screening tests are tools that have been developed to aid in determining the presence or absence of <i>M. avium</i> subsp. <i>paratuberculosis</i> within a herd. Animals found positive to these tests should be considered suspect unless they show clinical signs of Johne's disease (in which case they are considered positive) or they are confirmed positive or negative by an official Johne's disease test.
State	Any of the 50 States, the Commonwealth of Puerto Rico, the Commonwealth of the Northern Mariana Islands, the District of Columbia, and any territories and possessions of the United States.
State animal health official	The State official who is responsible for the livestock and poultry disease control and eradication programs in a State.
State Johne's Disease Group	A group of interested persons organized by the State animal health official to assist in the oversight and coordination of the State's Johne's program.
Susceptible species	Domestic and exotic ruminants, such as cattle, bison, sheep, goats, cervids, and camelids, which are capable of natural infection with <i>M. avium</i> subsp. <i>paratuberculosis</i> .
Test-negative herd	A herd that is enrolled in the program and meets the test-negative component requirements described in this document.
Test-negative level	Level 1, 2, 3, or 4 with each increase indicating a lower probability of Johne's disease in the herd.
Test-positive herd	A herd that is enrolled in the program and that meets the test-positive component requirements described in this document.
Test-positive level	Level A, B, C, or D; Level A indicates zero or an extremely low prevalence, and D indicates the highest prevalence of Johne's disease in the herd.
VS	Veterinary Services: The division of APHIS in charge of animal health activities within the United States.
USDA	U.S. Department of Agriculture.

Part II Administration

A. Designated Johne's Disease Coordinator (DJC)

1. General

Each State must have one person to act as its DJC. This person should be selected jointly by the State animal health official and the AVIC and be approved by the VS regional Johne's disease epidemiologist or regional director and the Johne's disease staff of APHIS, VS, National Animal Health Programs. During the initiation of the program in each State, States have a 1 year grace period to allow the chosen DJC candidate to meet the education and training requirements. During this period, the candidate is considered to be the acting DJC.

2. Qualifications. Each DJC candidate must

- a. Be a full-time State, Federal, or university veterinarian.
- b. Successfully complete a Johne's disease (JD) epidemiology course that includes onfarm risk assessments and development of herd management plans.
- c. Have at least 80 hours of experience in assessing risk, developing herd plans, and classifying JD test-positive animals and herds.

3. Responsibilities. The DJC has the responsibility to

- a. Interpret laboratory test results and classify animals and herds based on the use of official and screening tests.
- b. Provide training for State personnel performing program work.
- c. Provide training for Johne's certified veterinarians and develop a mechanism within the State to evaluate and monitor the involvement of the Johne's certified veterinarians.
- d. Review the risk assessments and herd management plans submitted by herd owners and Johne's certified veterinarians.
- e. Periodically audit the program to determine if it is adequately controlling JD in the State.
- f. Assist animal health officials, herd owners, and the herd owner's Johne's certified veterinarian with developing herd management plans as requested or needed.
- g. Participate in the program activities as a member of the State Johne's Disease Group.
- h. Provide a quarterly report to the VS regional Johne's disease epidemiologist and the Johne's staff of VS, National Animal Health Programs, on the progress of the program.

B. State Johne's Disease Group**1. General**

A Johne's disease group or an equivalent must be formed to assist the State in program development, implementation, and review. A representative at the producer level, for either the beef or the dairy industry, is recommended as the chairperson for the group. The DJC must be a member. The group must meet at least once a year.

2. Recommended members. This group should include, but not be limited to

- a. Dairy producers—purebred, commercial, and commodity groups
- b. Beef producers—purebred, commercial, and commodity groups
- c. University and extension faculty
- d. Animal health diagnostic laboratory personnel
- e. Regulatory veterinary medical officers—State, Federal, or field services, or all three
- f. Veterinary practitioners—beef and dairy

C. Johne's Certified Veterinarians**1. General**

States may elect to use the services of private practitioners in addition to State or Federal personnel to assist herd owners in conducting risk assessments and developing herd management plans. States using these veterinarians must determine that they meet the qualifications listed below. The DJC needs to develop a process to monitor the herd management plans developed by new Johne's certified veterinarians closely. For example, the DJC may require the new Johne's certified veterinarian to develop the first one-to-five herd management plans in conjunction with an experienced animal health official. Johne's certified veterinarians will be required to take a JD refresher course approved by the DJC at least once every 3 years.

2. Qualifications

Johne's certified veterinarians must be accredited veterinarians, must have received additional education on Johne's disease, and must be able to demonstrate to the DJC that they have the knowledge needed to

- a. Develop approved herd-management plans.
- b. Provide appropriate JD risk assessments.
- c. Understand JD epidemiology, testing, and test interpretation.
- d. Understand State and Federal program requirements.

- e. Collect and submit fecal, tissue, and blood samples for JD testing.

3. Responsibilities

Johne's certified veterinarians have the responsibility to

- a. Provide risk assessments and develop herd management plans that will meet the approval of the DJC when requested by the herd owners.
- b. Collect and submit samples according to the requirements set by the DJC.

Part III Program Elements and Procedures

A. Education

1. General

The education element in each State serves as the entry level for producers when participating in the State's voluntary program. The education element must provide producers with basic Johne's disease information, management strategies for controlling and eliminating the disease, and information on the various aspects of the State's program. Education can take place through group workshops or one-on-one sessions with the producer's veterinarian. A record of participation should be kept. In the education element of the program, producers should receive information on the topics indicated below.

2. Required topics for education

a. Basic JD information—cause, clinical stages, transmission, etc.

b. Management strategies for

- (1) Manure and waste handling
- (2) Colostrum and milk
- (3) Calves and young stock
- (4) Additions and high-risk animals
- (5) Biosecurity
- (6) Infected animals

c. Control and testing strategies:

- (1) Testing
- (2) Test interpretation

d. The State Program components

B. Management

1. General

Producers informed about Johne's disease may wish to participate in the management element, an intermediate step in the program. This level of participation recognizes producers for putting approved management practices and plans into place. At this stage, herd testing is an option available to the producer. The following components must be completed to the satisfaction of the DJC:

2. Risk assessment

Before developing an individual herd management plan, a Johne's certified veterinarian or an animal health official must conduct a risk assessment to identify aspects of management likely to spread *M. avium* subsp. *paratuberculosis* throughout the herd. A copy of the risk assessment must be submitted to the DJC with the herd management plan.

3. Herd management plan

The Johne's certified veterinarian or an animal health official, in conjunction with the herd owner, will develop a herd management plan to prevent the introduction of JD into the herd and to reduce transmission of the disease among animals within the herd. Clinical suspects should be segregated and diagnosed as soon as possible. Culture-positive cattle should be sent to slaughter or rendering. A copy of the herd management plan and risk assessment must be submitted to the DJC for review and final approval.

Guidelines for developing a herd management plan can be reviewed by reading "Prevention and Control of Johne's Disease in Dairy Herds: A Workbook for Veterinarians and Producers," first edition designed and edited by C. A. Rossiter, L. J. Hutchinson, D. Hansen, and R. H. Whitlock. This document can be found on the United States Animal Health Association's Web site at <<http://www.usaha.org/njwg/jddairy.html>>. The herd management plan should address management practices that prevent the calves and young stock from becoming infected with *M. avium* subsp. *paratuberculosis*. The herd management plan must discuss

- a. Animal identification—All cattle must be individually identified using an identification method approved by the State Johne's group. It is recommended that all animals in participating herds should be individually identified using an official eartag. Any previous regulations listed in Title 9 CFR regarding animal identification with other animal health programs still apply.
- b. Minimum biosecurity measures (to prevent bringing infection onto the farm)—These measures should be in place to reduce exposure to manure or milk from cattle of unknown JD status. Care is needed to prevent exposure to other susceptible species (e.g., sheep, goats, farmed deer, camelids, nonprogram cattle). The herd management plan should include the following biosecurity measures:
 - (1) Ensure that animals added to the herd come only from low-risk or known-status herds and from known sources (do not purchase from sale yards). Record the source and manage additions as higher risk animals unless you have evidence to the contrary.
 - (2) Minimize exposure of young stock to manure from adult animals, including other susceptible species. How the exposure is minimized will vary depending on management of the cattle located on the premises.
 - (3) Minimize exposure of livestock to susceptible animals that are infected or have been exposed to infected animals.
 - (4) Never feed calves unknown sources of colostrum. Never feed calves unknown sources of milk unless it is pasteurized.
 - (5) Minimize exposure of feed, water, equipment, and vehicles to manure.

c. Minimum management practices—dairy herds

- (1) Maternity area must be kept clean and dry and separate from other adult animals.
- (2) Each newborn calf must immediately be separated from adult animals.
- (3) Colostrum must be from a single identified cow; no pooled colostrum may be used.
- (4) Each calf must be fed colostrum from a test-negative or healthy low-risk animal.
- (5) Calves must be fed milk replacer or pasteurized milk.
- (6) Calves and heifers must be kept free from exposure to the manure of mature cattle and must be housed by age and separated from older animals.
- (7) Separate clinical suspects from maternity and young stock. Record all clinical suspects.

d. Minimum management practices—beef herds

- (1) Keep calving areas as clean and dry as possible.
- (2) Minimize the density of cow and calf pairs as much as possible.
- (3) Use feeding practices that reduce manure contamination of feed and feeding areas as much as possible.
- (4) Provide colostrum from the calf's dam or from another single source that is from a test-negative or healthy low-risk animal.
- (5) Raise weaned replacements, separated from older animals.

4. Renewal

To continue in the program, a herd owner and Johne's certified veterinarian must annually repeat the risk assessment and make appropriate changes to the herd management plan. The updated risk assessment and herd management plan must be submitted to the DJC.

C. Herd Testing and Classification

1. General

Herd testing and classification constitute the third program element. The purpose of this element is to recognize producers in the program publicly for putting approved management practices and plans into place as well as for separating test-negative herds from test-positive herds. Herds at this stage will continue

undergoing herd risk assessments and be subject to herd management plans that were developed under the management element. After initial testing, included herds may participate in either the test-positive or test-negative component of this element according to the test results.

2. Requirements for entrance

Herds enrolling in the herd testing and classification element must have completed a risk assessment and developed a herd management plan using the guidelines established in the management element.

a. Testing

Initial testing is required to determine the herd's test status. This can be accomplished by doing a screening test or an official Johne's disease test on a minimum of 30 randomly selected animals at second lactation (3 years old) or higher. In herds with fewer than 30 animals at second lactation or higher, first-lactation animals (2 years old) must also be included until 30 animals are tested or until all animals at first lactation and higher have been tested. Herd owners should be encouraged to test statistical subsets (see table 1—Sample Sizes for Subset Testing) or greater numbers of cattle when possible.

All samples must be collected by, or under, the supervision of an accredited veterinarian or a State or Federal animal health official. Vaccinated herds are eligible. Vaccinated herds must be tested by an organism-detection test. All samples must be submitted to a laboratory approved by NVSL.

b. Appealing the status of a test-positive animal

(1) For animals found positive to a screening test, a herd owner may elect to confirm the test results as follows:

- (a) An official Johne's disease test must be submitted by an accredited veterinarian within 45 days of notification of the screening test results.
- (b) If the official Johne's disease test is negative, the herd may retain its test-negative status, but that animal must be included in the next round of program testing if it remains in the herd.
- (c) If the animal that was test positive to a screening test has left the herd so that no confirmation of the results can be obtained, the DJC should conduct a risk assessment to determine the status of the herd.

- (2) To appeal positive results to an official Johne's disease test, a herd owner must submit a written statement to the DJC within 30 days of the positive results requesting an appeal, and then, at his or her own expense, arrange for an accredited veterinarian to
 - (a) Conduct a necropsy of the animal with culture and histopathology of the ileum and of the mesenteric and ileocecal lymph nodes; OR
 - (b) Conduct a full-thickness biopsy of the ileum and biopsy of the mesenteric or ileocecal lymph nodes with histopathology and culture of the tissues and a fecal culture sample taken at the time of biopsy; OR
 - (c) Submit six separate fecal cultures from the animal on samples collected between 30 and 45 days apart. All six cultures must be negative for the animal to be considered a test-negative animal.

The herd JD status will be suspended until all testing is completed. Only negative results on all tests will allow the herd to retain a test-negative status.

(3) Placement

Herds that test positive must remain in the management element or be enrolled in the test-positive component. Herds that test negative will be eligible to enter the test-negative component.

3. Test-positive component (Control and Testing)

The purpose of the test-positive component is for the State to maintain a record of infected herds that are being tested, with approved risk assessment and herd management plans in place. States may include assessment levels for herd prevalence. Herds enrolled in this component must use testing protocols approved by the DJC.

a. Requirements for herds in the test-positive component

- (1) Application—The herd owner enrolling the herd must sign an agreement to abide by the requirements concerning minimum biosecurity and management, identification, testing, and herd addition strategies. This agreement must be renewed every 10 to 14 months from the anniversary date.
- (2) Herd additions
 - (a) All purchased animals should be from herds with approved herd management plans.

- (b) Heifers raised off the premises should be raised with the minimum biosecurity and control measures in place.
- (3) Testing—The herd owners along with the Johne's certified veterinarian must develop a testing protocol as follows:
 - (a) All samples must be collected by, or under, the supervision of an accredited veterinarian or a State or Federal animal health official.
 - (b) Vaccinated animals must be tested using an official Johne's disease test.
 - (c) All samples must be submitted to a laboratory approved by NVSL.
 - (d) All animals specified in the test protocol must be tested within 10 to 14 months of the anniversary date.
 - (e) Herds not adhering to the prescribed testing requirements will be placed in the management element.
 - (f) It is recommended that animals with positive results on an official Johne's disease test be identified as infected and restricted to the premises. When infected cattle leave the herd, it is recommended that they go directly to slaughter or rendering.
 - (g) A test-positive animal status may be appealed using the same appeal process described above for entry into the herd testing and classification element. If an animal is removed from the herd while screening test results are pending, a fecal culture should be collected, submitted, and held at the laboratory. This will allow the owner to appeal the herd level if the animal tests positive to a screening test. Final classification of the animal and herd will be made by the DJC.

(4) Optional—Assessment levels

The State's test-positive component may use assessment levels. Herds in a State program may achieve Level A, B, C, or D. Each level classifies a herd based on the apparent prevalence of Johne's disease within the herd. The level achievement year should also be indicated. For example, a herd that completed Level B testing in 2001 and elects to remain at Level B in 2002 would have a Level B 2001 status. The level achievement year should be noted because continued monitoring increases confidence that the herd prevalence is within that category. States that use assessment levels must follow the test-positive level requirements listed next.

- (a) Level A—An annual herd test reveals no screening or official Johne's test-positive animals. Level A can be maintained by achieving negative screening test results on 30 second- or higher lactation animals every 10–14 months. Herds achieving Level A should be encouraged to enter the test-negative program at Level 1. Qualifying herds have

Negative test results on at least 30 randomly selected second- or higher lactation animals (3 years old or older),

OR

Negative test results on the whole herd and on bulls over 2 years of age.

- (b) Level B—An annual whole-herd test with the addition of bulls over 2 years of age reveals less than 5 percent of animals positive to a screening or official Johne's disease test.
- (c) Level C—An annual whole-herd test with the addition of bulls over 2 years of age reveals at least 5 percent, but not more than 15 percent, of animals positive to a screening or official Johne's disease test.

- (d) Level D—Herds should be classified as Level D if either of the following apply:

A test on at least 30 randomly selected second- or higher lactation animals (3 years old or older) reveals one or more test-positive animals,

OR

A whole-herd test with the addition of bulls over 2 years of age reveals more than 15 percent of the animals positive to a screening or official Johne's disease test.

b. Renewal and Advancement

A herd will remain in this classification for up to 14 months. For continuation, the herd owner must reapply with a copy of the test results, updated herd management plan, and an agreement to follow the test-positive component requirements. If the herd owner wishes to renew or advance but is waiting for confirmation of screening test results and therefore cannot meet the 14-month deadline, he or she can send the DJC a letter of intent to renew or advance. If the letter of intent is received within 30 days after the deadline, the DJC may allow the herd to retain its status for up to 5 months. Herds for which the necessary test results have not been supplied by the 5-month deadline must be placed in the management element. Owners of removed herds may reapply after the herds complete the required testing.

4. Test-negative component (Herd Status)

The test-negative component must include a herd management plan based on the requirements in the management element. The test-negative component includes a series of levels; owners may improve their herd status (achieve a higher level) by additional testing and biosecurity measures. Each higher level represents a greater probability that the herd is free of JD. However, this does not certify that a herd is free of JD. Herds in the test-negative component may remain at any given level by doing monitoring testing or may advance to a greater surveillance level with additional testing. Requirements for herds in the test-negative component include

a. Application

The herd owner enrolling the herd must sign an agreement to abide by the requirements concerning minimum biosecurity and management established in the management element as well as the identification, testing, and herd-addition requirements listed below.

b. Herd Advancement—Test negative

Test-negative herds may achieve Level 1, 2, 3, or 4. Each higher level represents a greater probability that the herd is free from JD. However, this does not certify that a herd is free of JD. The level achievement year should also be indicated. For example, a herd completing Level 2 testing in 2001 and electing to remain at Level 2 in 2002 would have a Level 2 2001 status. The level achievement year should be noted because continued monitoring increases confidence the herd is not infected. To advance from one level to the next, a statistical subset must be tested (table 1—Sample Sizes for Subset Testing), and the herd must meet the level requirements listed below for standard track or fast track test-negative component levels.

(1) Standard Track—The standard track is designed to allow entry into the program with a minimal investment of funds and gradually increase the producer's investment in the program. The standard track will require at least 3 years and four tests to reach Level 4.

- (a) Level 1—The herd owner has developed a herd management plan and has agreed to abide by the requirements of the test-negative component, and the herd has had negative screening test results on 30 second- or higher lactation animals.
- (b) Level 2—Herds have met the requirements for Level 1 and have had negative screening tests on a statistical subset of second- or higher lactation animals. Level 2 testing must be completed within 10 to 14 months of any Level 1 testing.

- (c) Level 3—Herds have met the requirements for Level 2 and have had negative fecal culture results on a statistical subset of second- and higher lactation herd members. Bulls 2 years of age and older must be included in this testing. The fecal culture must be collected within 10 to 14 months of any Level 2 testing.
 - (d) Level 4—Herds have met the requirements for Level 3 and have had a negative screening test on a statistical subset of second- or higher lactation animals. Level 4 testing must be completed within 10 to 14 months of any Level 3 testing.
- (1) Fast Track—The fast track allows producers to proceed to a higher level of confidence more quickly than the standard track but requires greater financial investment at program entry. The fast track will allow herds to reach Level 4 in 2 years with three tests.
- (a) Level 1—Skip this level if owner signs a declaration that no cows were seen or diagnosed with Johne's disease in the past 5 years and has an approved herd plan in place. The State may require the declaration to be cosigned by the herd veterinarian. The signed declaration must include the following statements:
 - I am fully aware of the management and disease history of the herd during the past 5 years.
 - Johne's disease is not known or suspected to have existed in the herd during the past 5 years or on the property during the past 12 months.
 - No cattle have been introduced from known infected herds during the past 5 years.
 - (b) Level 2—Herds have met requirements for Level 1 and have had a negative screening test on a statistical subset of second- or higher lactation animals. Level 2 testing must be completed within 10 to 14 months of any Level 1 testing.
 - (c) Level 3—Herds have met the requirements for Level 2 and have had negative fecal culture results on 30 second- or higher lactation cows and all bulls 2 years or older. The fecal culture must be collected within 10 to 14 months of any Level 2 testing.
 - (d) Level 4—Herds have met the requirements for Level 3 and have had a negative screening test on a statistical subset of second- or higher lactation animals. Level 4 testing must be completed within 10 to 14 months of any Level 3 testing.

c. Animal identification

All test-eligible animals must be individually identified using an official eartag. Any previous regulations listed in 9 CFR regarding animal identification for any other APHIS program still apply.

d. Testing

- (1) All samples must be collected by, or under, the supervision of an accredited veterinarian or a State or Federal animal health official.
- (2) Vaccinated herds will be eligible for the test-negative component after vaccination has been discontinued. All testing must be done by an official Johne's disease test until enough nonvaccinated natural additions qualify for serology testing. The number of nonvaccinated animals will be the sample size required for that size herd for a statistical subset for serology.
- (3) All samples must be submitted to a laboratory approved by NVSL.
- (4) Herd removal provisions—If an animal in a test-negative herd tests positive to an official Johne's disease test, or if the testing requirements are not followed, the herd must be removed from the test-negative component and placed in the test-positive component or in the management element (unless an appeal is pending). Animals that test positive to a screening test must be confirmed with an official JD test or their status will be removed or suspended.
- (5) Appealing the status of a test-positive animal must be done using the same appeal process as for entry into herd classification and testing.

e. Herd additions

- (1) Purchased heifers and bulls less than 2 years of age may be added to the herd, provided that:

The animal was purchased from a herd with a test-negative level that is equal to or higher than the herd it is entering,

OR

The purchased animal is tested in the next herd test after it reaches 2 years of age. Herds containing animals purchased from herds with a lower or unknown status will be restricted to Level 1 until all additions reach test-eligible age. At that time, herds may continue in the test-negative component by following the testing protocol for Level 2.

- (2) Purchased or replacement animals 2 years of age or older may be added to the herd provided that

For test-negative Level 1, 2, or 3 herds:

The animal was purchased from a herd with a test-negative level equal to or higher than the herd it is entering,

OR

The animal has a screening test within 30 days before entering the test-negative level herd with negative results,

AND

Fecal cultures have been collected from each animal added and submitted between 30 days before and 30 days after arrival,

AND

The herd addition is tested on the next herd test. The addition's status will remain at its entry level until it has tested negative at the next herd test.

For test-negative Level 4 herds:

The animal was purchased from a herd with a test-negative level that is equal to the herd it is entering,

OR

The herd of origin has a test-negative level of 2 or 3 and the purchased addition has a screening test with negative results within 30 days before entry into the program herd,

AND

Fecal cultures have been collected from each animal added and submitted between 30 days before and 30 days after arrival,

AND

The herd addition is tested on the next herd test. The addition's status will remain at its entry level until it has tested negative at the next herd test.

- (3) Heifers raised off the premises must be raised with the proper biosecurity measures in place and with animals at an equal or greater test-negative level.
- (4) Test-negative program herds may use semen and embryos from other cattle herds, provided that the semen used is processed according to Certified Semen Services' standards and the embryos are processed according to International Embryo Transfer Society protocols. Embryo transfer recipient cows must meet herd addition requirements.

f. Herd Level Maintenance—Test negative

All levels can be maintained by achieving negative screening test results on 30 second- or higher lactation animals every 10 to 14 months. The level achievement year should continue to be noted because continued monitoring increases confidence the herd is not infected.

g. Renewal and Advancement

A herd will remain at any level for up to 14 months. For continuation of this classification, the herd owner must reapply with a copy of negative test results, an updated herd management plan, and an agreement to follow the test-negative component requirements. If a herd owner wishes to renew or advance but is waiting for confirmation of screening test results and therefore cannot meet the 14-month deadline, he or she can send the DJC a letter of intent to renew or advance. If the letter of intent is received by 30 days after the deadline, the DJC may allow the herd to retain its status for up to 5 months. Herds for which the necessary test results have not been supplied by the extended 5-month deadline must be removed from the test-negative component. Herds removed from the test-negative component may reapply at the test-negative Level 1.

Part IV - Laboratory Procedures

A. Approved Laboratories

1. General

All official Johne's disease and screening tests used for the program may be conducted in a private, university, State, or Federal laboratory that has been specifically approved for conducting JD testing. The State animal health official has the authority to decide if private laboratories may participate in the program. States must have the authority to audit the JD diagnostic laboratories participating in the program periodically. If a laboratory lies outside of the State, the State may rely on audits conducted by the animal health officials from the State in which the laboratory is located.

2. Approval process for laboratories performing official Johne's disease tests

- a. A laboratory seeking approval to perform official Johne's disease tests must contact NVSL for a test kit of 25 samples. (A valid check test sample from NVSL will be determined by a consensus of at least 70 percent of the participating laboratories.)
- b. The laboratory must correctly identify 100 percent of the negative test samples.
- c. The laboratory must correctly identify 100 percent of the Too Numerous To Count (TNTC) test samples.
- d. The laboratory must correctly identify at least 70 percent of the test samples that were not classified as negative or TNTC.
- e. The laboratory must use the same procedure and materials during the check test as are used during routine testing.
- f. No retest is available within the same fiscal year.

3. Approval process for laboratories performing screening tests (serology tests)

- a. A laboratory seeking approval to perform screening tests must contact the NVSL for a test kit of 25 samples. (A valid check sample will be determined by NVSL using available licensed ELISA kits.)
- b. The laboratory must correctly identify at least 90 percent of the serology check test samples.
- c. The laboratory must use the same procedure and materials during the check test as are used during routine testing.
- d. One retest is available if a laboratory fails the first time.

B. Approved program tests

1. Official Johne's disease tests
 - a. Fecal and tissue culture—Culture is the standard for organism-based tests, although culture methods are not currently standardized. Methods include both solid and liquid culture preparations. Protocols for recommended methods can be obtained from NVSL upon request. Sensitivity is estimated at 40 ± 10 percent; specificity is considered to be 99 percent.
 - b. DNA probe—DNA probes can detect the presence of *M. avium* subsp. *paratuberculosis* without having to grow it. The test has the advantage that it takes less than 3 days but has the disadvantages of higher cost and the potential of missing low shedders. Sensitivity is estimated at 40 percent; specificity is about 99.9 percent.
 - c. Histology of tissue—**No check test is available at this time.** Microscopic identification of the characteristic pathological changes and of *M. avium* subsp. *paratuberculosis* organisms in tissue is a definitive test for JD. Tissue changes and bacteria can be observed in the intestinal lining and in nearby ileal, mesenteric, and ileocecal lymph nodes in infected animals. Sensitivity depends on the stage of disease and the number and type of specimens collected but is typically considered to be greater than other laboratory tests.
2. Screening test
 - a. USDA-approved enzyme-linked immunosorbent assay (ELISA)—Animals found positive with ELISA tests should be considered suspect until confirmed using an official Johne's disease test. ELISA tests are to be used as screening tools or for helping make management decisions. Sensitivity has been estimated at 25 percent for nonclinical cases and approximately 85 percent for clinical cases; specificity is between 98 and 99 percent.

Appendix

Table 1—Sampling sizes for subset testing

Number of cattle in herd (2d or higher lactation)	Number of cattle to sample (2d or higher lactation)	
	<i>ELISA</i>	<i>Fecal culture</i>
≤ 300	Test all.	Test all.
301–400	Test all.	Test all (up to 313).
401–500	Test all.	324
501–600	Test all (up to 531).	332
601–700	540	338
701–800	547	342
801–900	552	345
≥ 901	580	360

In smaller herds, all cattle of second or higher lactation must be tested. In herds with fewer than 30 second- or higher lactation animals, first-lactation animals must also be tested.

Sample numbers above are based on the following assumptions:

- The cattle to be tested are in second or higher lactation.
- ELISA tests are assumed to have 25-percent sensitivity and fecal cultures are assumed to have 40-percent sensitivity. (These were consensus estimates of the Herd Status Committee of the National Johne's Working Group, United States Animal Health Association, for subclinically infected cows in first or higher lactation, and no changes were made for older populations sampled.)
- ELISA and fecal culture are assumed to have 100-percent test specificity (given followup of all ELISA positives with fecal culture).
- The confidence of detecting infection (at least 1 test-positive cow), if present at a true prevalence of 2 percent, is 95 percent.
- The calculations are based on sampling without replacement.

